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Effect of Starvation on Some Glycolytic Enzymes in Adult *Isoparorchis hypselobagri* (Billet, 1898) (Trematoda: Digenea)

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Abstract: The enzymatic assays made on extracts of the adult fluke *Isoparorchis hypselobagri* demonstrated the presence of hexokinase (HK, EC 2.7.1.1), glycogen phosphorylase (GP, EC 2.4.1.1), phosphoglucose isomerase (PGI, EC 5.3.1.9) and lactate dehydrogenase (LDH, EC 1.1.1.27). In the starved fluke all the studied glycolytic enzymes are depleted except glycogen phosphorylase-a activity, which shows increasing trend.

Key words: *Isoparorchis hypselobagri*, glycolytic enzymes, phosphorylase, starvation

INTRODUCTION

The pathways of carbohydrate metabolism have been investigated in detail in several parasitic helminthes. In parasitic trematode, carbohydrate initially is broken down by glycolysis (Barrett, 1981; Smyth, 1994; Barrett and Precious, 1995). However, no information on the enzymes of glycolytic and glycogenolytic pathway in *in vitro* starvation period is available in *Isoparorchis hypselobagri*. Adult *Isoparorchis hypselobagri*, a piscine digenetic trematode harbors within the swimbladder of *Wallago attu*, an oxygen rich habitat. The principal objective of the present study is to know the *in vitro* starvation effects on some glycolytic and glycogenolytic enzymes, hexokinase (HK, EC 2.7.1.1), glycogen phosphorylase-a (GP, EC 2.4.1.1), phosphoglucose isomerase (PGI, EC 5.3.1.9) and lactate dehydrogenase (LDH, EC 1.1.1.27) and assessment of their role in anaerobic glycolysis in this fluke.

MATERIALS AND METHODS

Live flukes were recovered from the swim bladder of *Wallago attu*, obtained from the local fish market as well as from the fishermen at Kangsabati reservoir, Mukutmanipur, Bankura, India. All the experimental works were done in the Parasitology Laboratory, Department of Zoology, University of Calcutta. The flukes just after collection were subjected to starvation for 6, 12, 18, 24, 36, 48, 60 and 72 h in phosphate buffered saline (PBS, pH 7.0; Taylor and Baker, 1978) without glucose. The parasites were maintained alive under aerobic condition at room temperature (30±1°C) in PBS solution with 250 mg Streptomycin and 100000 Unit Penicillin per 100 mL (Srivastava and Gupta, 1977) to prevent bacterial contamination.

A single fluke after taking weight in a semi-micro balance (ADA, 71 L^{-1}), was homogenized in 2 mL ice-cold 0.1 M tris-HCl buffer (pH 7.4) using an all glass Potter-Elvehjem homogenizer at 4°C (Yusufi and Siddiqi, 1978). The samples were centrifuged at 10,000 rpm at 0-4°C for 30 min to remove the cell debris. The supernatant thus obtained was used in the enzyme assay.

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Specific activity of hexokinase (HK, EC 2.7.1.1) was expressed as nmoles of G-6 P produced per minute per mg protein (Joshi and Jagannathan, 1966). Glycogen phosphorylase (GP, EC 2.4.1.1) activity was estimated as the amount of inorganic phosphorous released when the enzyme was added to α-D-glucose-1-phosphate in presence of glycogen (Platzer and Roberts, 1970). Inorganic phosphate was measured following the method of Fiske and Subbarow (1925). Phosphoglucose isomerase (PGI, EC 5.3.1.9) is measured by the Seliwanoff reaction where ketohexose/ketohexose phosphate react with resorcinol-thiourea (Roe *et al*., 1949). Specific activity of lactate dehydrogenase (LDH, EC 1.1.1.27) is expressed as nmoles of NADH oxidized per minute per mg protein (Kornberg, 1955). Protein was estimated following the method of Lowry *et al*. (1951). Molar decadic absorption co-efficient ($1\times mol^{-1}\times mm^{-1}$) for NADH and NADPH at temperature 25 and 30°C are taken for practical use (NADH 6.3×10^2 at 340 nm) (Bergmeyer, 1974).

In each experiment ten parasites were taken. Data collected on each specimen is recorded, categorical variables are analysed and the results are expressed as mean±SEM (standard error of mean). Statistical probability is calculated using student t-test.

RESULTS AND DISCUSSION

The specific activity of HK and LDH are high in fresh control fluke than GP-a and PGI. The specific activity of GP-a, a glycogenolytic enzyme, is very much low in fresh control fluke and varies between 3.47 to 6.61 nmol min^{-1} mg^{-1} protein with an average value of 5.24±1.08 nmol min^{-1} mg^{-1} protein. But after starvation period of 72 h it is increased to 77.53±1.61 nmol min^{-1} mg^{-1} protein. However, all the enzymes except GP-a show gradual decline during *in vitro* starvation. The LDH, an important enzyme of glycolytic pathway shows reduced activity after 72 h of starvation is 21.72±0.96 nmol min^{-1} mg^{-1} protein (Table 1).

The present investigation deals with the enzyme activity during glycolysis (hexokinase, phosphoglucose isomerase, lactate dehydrogenase) and glycogenolysis (glycogen phosphorylase-a) in the fresh-control and in *in vitro* starved *I. hypselobagri*. The hexokinase (HK) is an important regulatory enzyme as it indicates phosphorylation of glucose via glycolysis and pentose phosphate pathway and activates the formation of glycogen and complex carbohydrates from glucose (Barrett, 1981; Smyth and Mc Manus, 1989; Smyth, 1994). Hexokinase (HK) phosphorylates glucose to glucose-6-phosphate with the help of ATP, Mg^{2+}. The enzyme transfer the phosphate group of the Mg^{2+}-ATP complex to C^6-hydroxyl group of glucose producing Mg^{2+}-ADP and glucose-6-phosphate. The reaction is practically irreversible in the physiological condition. In the present finding the specific activity of HK in the starved fluke decreases significantly than the fresh control fluke. The average specific activity of HK in the fresh control fluke, 101.95±0.97 nmole min^{-1} mg^{-1} protein is comparable to the HK activity of *Srivastavaia indica*, 1.41±0.08 μmol min^{-1} mg^{-1} protein and

Table 1: Levels of specific activities of some glycolytic enzymes in adult *Isoparorchis hypselobagri* during *in vitro* starvation

Study groups	HK (nmol min^{-1} mg^{-1} protein)	GP-a (nmol min^{-1} mg^{-1} protein)	PGI (nmol min^{-1} mg^{-1} protein)	LDH (nmol min^{-1} mg^{-1} protein)
Control (10)	101.95±0.97	5.24±1.08	29.78±0.50	104.23±1.23
6 h (10)	97.74±0.76**	6.98±0.91**	28.82±0.43**	99.65±1.25**
12 h (10)	89.56±0.79**	11.78±1.10**	27.83±0.43**	94.84±1.13**
18 h (10)	77.52±0.70**	16.62±1.63**	26.90±0.29**	85.84±1.19**
24 h (10)	65.82±0.79**	21.54±1.57**	25.77±0.30**	77.65±1.32**
36 h (10)	66.70±0.71**	30.56±1.68**	24.30±0.30**	65.85±1.25**
48 h (10)	69.45±0.61**	41.95±1.51**	23.17±0.33**	45.72±1.03**
60 h (10)	47.66±0.79**	58.73±1.90**	21.87±0.38**	31.50±0.97**
72 h (10)	20.79±0.73**	77.53±1.61**	19.95±0.29**	21.72±0.96**

Results are expressed as ±SEM (Standard Error of Mean); Figure in the parentheses indicates the number of parasite studied; **: $p<0.01$ is highly significant

Gastrothylax crumenifer, 1.07±0.08 μmol min^{-1} mg^{-1} protein (Yusufi and Siddiqi, 1978) and shows greater values than the present fluke. The quantitative data on specific activity of HK is also available from several helminthes; 2 nmol min^{-1} mg^{-1} protein in *Mesocystoides corti* (Kohler and Hanselmann, 1974), 3 nmol min^{-1} mg^{-1} protein in *Echinococcus granulosus* and 4 nmol min^{-1} mg^{-1} protein in *E. multilocularis* (Mc Manus and Smyth, 1982), 10 nmol min^{-1} mg^{-1} protein in *Schistocephalus solidus* (Korting and Barrett, 1977) and 6 nmol min^{-1} mg^{-1} protein in *Ligula intestinalis* (Mc Manus and Sterry, 1982). In the present finding, HK activity decreases significantly in the starved flukes than in the fresh control fluke. The rate of glycolysis decreases at lower pH (Kapur *et al*., 1985; Sen, 2002) and also inhibited by its product glucose-6-phosphate in a cestode *Hymenolepis diminuta* (Komuniecki and Roberts, 1977) and in trematode *Schistosoma mansoni* (Barrett, 1981). It is also reported that the HK reaction is also inhibited by an elevation of 2,3-diphosphoglyceraldehyde concentration (Sen, 2002). Both mammalian hexokinase and the glucokinase of *S. mansoni* are inhibited by high levels of ADP, but this is not thought to be physiologically significant (Barrett, 1981). Thus, the HK reaction is decreased or inhibited during starvation period as observed here may be due to increase in the level of glucose-6-phosphate or 2,3-DPG. Another probable cause may be the energy yielding process in the fluke through the metabolism of glucose is depleted in *in vitro* starvation time. During *in vitro* starvation the consumption of glucose decrease in the fluke (Bera *et al*., 2002).

Glycogen phosphorylase exists in two forms in muscles (i) phosphorylase-b (GP-b) is inactive except at high concentration of AMP and Pi and carries no phosphoserine group, (ii) phosphorylase-a (GP-a), is active even in absence of AMP but carries phosphoserine residues. Phosphorylase-b is changed to phosphorylase-a through the phosphorylation of serine residues by ATP and phosphorylase kinase. The specific activity of GP-a is increased significantly in the starved fluke with increase of starvation time than the fresh-control fluke where GP-a activity is very much low. The reports on GP-a activity is restricted in limited flukes. In *Schistosoma mansoni*, GP exists in inactive and active forms and there is evidence for the interconversion of these two forms (Bueding and Fisher, 1982) and is also stimulated by AMP. The GP of *Fasciola hepatica* also exists in active and inactive forms; activation is stimulated by 3′,5′-cyclic AMP and the activity of the enzyme increased by AMP (Mansour and Mansour, 1979; Simonic and Locatelli, 1978). The cysticercoids of *Hymenolepis diminuta* have a and b forms of phosphorylase and their interconversion similar to the situations in mammals, is regulated by a 3′,5′-cyclic AMP-dependent protein kinase and a phosphorylase phosphatase (Moczon, 1975, 1977). The GP performs a role similar to that of HK and controls in activation of glycogen for synthetic reaction and in metabolism to give glucose-1-phosphate, which enters the glycolytic sequence.

Glycogen is broken down during *in vitro* starvation of the fluke (Bera *et al*., 2002) which is accompanied by a significant increase in the GP-a activity. This suggests that, the energy demand by the trematode parasite was probably enhanced under the test conditions leading to stimulation of glycogenolysis and inhibition of gluconeogenesis. The increase in GP-a activity in the present fluke suggests the higher rate of glycogen depletion. Another probable cause may be that, the parasitic flukes normally lives on host's glucose supply, so there is no need for use of its reserve energy store. During starvation it starts to utilize its glycogen store through the activation of phosphorylase even before the complete depletion of glucose. As the starvation continues it starts to synthesize more and more phosphorylase, part of which are converted to the activation of enzyme.

Phosphoglucose isomerase (PGI) is another glycolytic enzyme, which isomerizes glucose-6-phosphate to fructose-6-phosphate. It is thus an aldose-ketose isomerase, changing the aldose glucopyranose to the ketose fructofuranose. The average specific activity of PGI in the fresh control fluke in the present finding is 29.78±0.50 nmol min^{-1} mg^{-1} protein, which is variable with *Gastrothylax crumenifer* as 55.7±4.0 nmol min^{-1} mg^{-1} protein and *Srivastavaia indica* as 124.9 nmol min^{-1} mg^{-1} protein (Yusufi and Siddiqi, 1978), but in the present study it is much more

lower. The higher PGI activity is reported in the protoscoleces of *Echinococcus granulosus* and *E. multilocularis* is 6964 nmol min^{-1} mg^{-1} protein and 3321 nmol min^{-1} mg^{-1} protein (Mc Manus and Smyth, 1982) respectively, in plerocercoids of *Schistocephalus solidus* is 559 nmol min^{-1} mg^{-1} protein (Korting and Barrett, 1977) and in plerocercoid of *Ligula intestinalis* 5218 nmol min^{-1} mg^{-1} protein (Mc Manus and Sterry, 1982). So, it is found that the levels of PGI activity is variable from species to species and also to the developmental stages of different species.

The specific activity of PGI in adult *I. hypselobagri* is reduced during *in vitro* starvation period. It is reported that 2,3-DPG acts as a nondiffusible intracellular anion and in the plasma pH (Kapur *et al*., 1985; Sen, 2002) and this might results in the decrease in the specific activity of PGI. It has also been reported that the glycogenolysis and anaerobic glycolysis are stimulated, leading to accumulation of lactate and H^+ ions and results in intracellular acidosis. Finally, the accumulation of proteins, lactate and NADH leads to inhibition of glycolysis and anaerobic energy production (Sen, 2002). All these factors may be involved resulting in inhibition of glycolysis which is reflected in the lowering of specific activity of PGI observed in the present study.

Lactate dehydrogenase (LDH) is a glycolytic oxidizing enzyme responsible for the reduction of pyruvate to lactate and oxidation of lactate to pyruvate. The study of this enzyme is very important in determining whether an animal is aerobic or anaerobic. Generally, under aerobic conditions the activity of the LDH is high and lactate is oxidized to pyruvate, so that it undergoes decarboxylation to form acetyl Co-A and then incorporates into the Krebs cycle. But under anaerobic conditions as in parasitic helminths the LDH activity is low and cytoplasmic redox balance is maintained through malate dehydrogenase (MDH) and reduction of oxaloacetate to malate. Oxidation of lactate to pyruvate and its subsequent incorporation into the Krebs cycle may not take place. The LDH activity reported earlier in trematode species are in *Dicrocoelium dendriticum*, *Fasciola hepatica*, *Schistosoma mansoni* (Von Brand, 1973), *Gastrothylax crumenifer* and *Srivastavaia indica* (Yusufi and Siddiqi, 1978) and *Fasciola hepatica* and *F. gigantica* (Prichard and Schofield, 1968; Umezurike and Anya, 1980). The LDH activity in *I. hypselobagri* is 104.23±1.23 nmol min^{-1} mg^{-1} protein, which is comparable with *Gastrothylax crumenifer* as 49.9±3.2 μmol min^{-1} mg^{-1} protein and *Srivastavaia indica* as 83.5±1.7 μmol min^{-1} mg^{-1} protein (Yusufi and Siddiqi, 1978). The low levels of LDH activity have also been reported in *F. hepatica* and *F. gigantica* (Prichard and Schofield, 1968), where anaerobic carbohydrate metabolism occurs (Smyth, 1994; Umezurike and Anya, 1980), although higher activity of LDH was also reported from schistosomes (Bueding and Saz, 1968) which depends on anaerobic energy metabolism (Symth, 1994). In the present fluke, the LDH activity is low and during *in vitro* starvation LDH activity decreases further. So, it may be considered that in this parasite anaerobic energy metabolism occurs, because during *in vitro* starvation the oxygen consumption by the fluke is decreased (Siddiqi and Nizami, 1975). The reasons why flukes show differences in their carbohydrate metabolism is not clear but Coles (1975) suggested that it might be due to their different host specificity. The quantitative data on LDH activity in several other helminths are also available, where anaerobic type of energy metabolism occurs. In *Mesocestoides corti* the LDH activity is 102 nmol min^{-1} mg^{-1} protein (Kohler and Hanselmann, 1974), in *Echinococcus granulosus* and *E. multilocularis* 611 and 652 nmol min^{-1} mg^{-1} protein (Mc Manus and Smyth, 1982) respectively, in *Schistocephalus solidus* 320 nmol min^{-1} mg^{-1} protein (Korting and Barrett, 1977) and in *Ligula intestinalis* 351 nmol min^{-1} mg^{-1} protein (Mc Manus and Sterry, 1982). As the parasite under study is rich in stored carbohydrate, especially glycogen (Bera *et al*., 2002), it seems that glycogenolysis may operative in *in vitro* starvation as shown by the increasing trend of glycogen phosphorylase-a. The low value and decreasing trend of LDH activity in *in vitro* starvation period reflects the anaerobic breakdown of carbohydrates in this fluke. Furthermore, the presence of HK, GP-a, PGI and LDH indicates that the breakdown of carbohydrate in this trematode is taking place through the classical Embden-Meyerhof pathway.

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